84/345



$$\begin{array}{c} (\operatorname{Fuc})_{i} \\ \mathbf{A} \longleftarrow \operatorname{GlcNAc} - \operatorname{GlcNAc} -$$

a-d, i, r-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 or 1. n, v-y = 0; z = 0 or 1; R = polymer

FIG. 29A

```
CHO, BHK, 293 cells, Vero expressed IF-beta h=1 to 3;
a-g, j-m, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
n, v-y = 0; z = 1.
```

```
    Sialidase
    CMP-SA-PEG, ST3Gal3
```

```
h = 1 to 3;
a-g, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.
```

FIG. 29B

```
Insect cell expressed IF-beta
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0 or 1;
z = 1.

1. GNT's 1&2, UDP-GIcNAc
```

2. Galactosyltransferase, UDP-Gal

2. CMP-SA-PEG, ST3Gal3, buffer, salt

```
b, d, f, h, k, m, n, s, u, w, y = 0;
a, c, e, g, i, r, t (independently selected) = 0 or 1;
j, l, v, x (independently selected) = 0 or 1;
z = 1; R = PEG.
```

FIG. 29C

86/345

```
Yeast expressed IF-beta
a-n=0; z=1;
r-y (independently selected) = 0 to 1;
R (branched or linear) = Man, oligomannose or
polysaccharide.
```

- 1. Endo-H
- 2. Galactosyltransferase, UDP-Gal
- 3., CMP-SA-PEG, ST3Gal3

```
a-m, r-z= 0; n = 1; R' = -Gal-Sia-PEG.
```

FIG. 29D

```
h = 1 to 3;
a-g, j-m, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
n, v-y = 0; z = 1.

1. CMP-SA-PEG, ST3Gal3

h = 1 to 3;
a-g, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.
```

CHO, BHK, 293 cells, Vero expressed IF-beta

FIG. 29E

```
\label{eq:constraints} \begin{split} &\operatorname{Insect\ cell\ expressed\ IF-beta}\\ &a-d,\,f,\,h,\,j-n,\,s,\,u,\,v-y=0;\ e,\,g,\,i,\,r,\ t\\ &(\operatorname{independently\ selected})=0\ or\ 1;\ z=1. \end{split}
```

```
    GNT's 1,2,4,5, UDP-GlcNAc
    Galactosyltransferase, UDP-Gal
    CMP-SA-PEG, ST3Gal3
```

```
a-m, r-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.
```

FIG. 29F

```
Yeast expressed IF-beta

a-n = 0; z = 1;

r-y (independently selected) = 0 to 1;

R (branched or linear) = Man, oligomannose.
```

```
    mannosidases
    GNT's 1,2,4,5, UDP-GlcNAc
    Galactosyltransferase, UDP-Gal
    CMP-SA-PEG, ST3Gal3
```

```
a-m, r-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.
```

```
NSO expressed IF-beta
a-i, r-u (independently selected) = 0 or 1;
j-m, n, v-y = 0; z = 1.
```

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H₄N₂-PEG

a-i, j-m, r-y (independently selected) = 0 or 1; n = 0; z = 1; R = PEG.

FIG. 29H

```
CHO, BHK, 293 cells, Vero expressed IF-beta h=1 to 3;
a-g, j-m, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
n, v-y = 0; z = 1.
```

1. CMP-SA-PEG, α2,8-ST

```
h = 1 to 3;
a-g, i, r-u (independently selected) = 0 or 1;
j-m (independently selected) = 0 to 2;
v-y (independently selected) = 1,
when j-m (independently selected) is 2;
z=1; n=0; R=PBG.
```

FIG. 291

CHO, BHK, 293 cells, Vero expressed IF-beta a-g, j-m, r-u (independently selected) = 0 or 1; h=1 to 3; n, v-y=0; z=1.

- 1. Sialidase
- 2. Trans-sialidase, PEG-Sia-lactose

a-g, j-m, r-y (independently selected) = 0 or 1; h = 1 to 3; n = 0; z = 1; R = PEG.

FIG. 29J

CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h=1; n, v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3
- a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n=0;
- v-y (independently selected) = 0 or 1; R = PEG.

90/345

```
NSO expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n, v-y = 0; Sia (independently selected) = Sia or Gal.
```

1. Sialidase and α-galactosidase
2. α-Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

```
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; R = PEG
n = 0; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
```

FIG. 29L

```
CHO, BHK, 293 cells, Vero expressed Ifin-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n, v-y = 0.
```

```
    Sialidase
    CMP-SA-PEG (16 mol eq),
ST3Gal3
    CMP-SA, ST3Gal3
```

```
a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n = 0; v-y (independently selected) = 0 or 1; R = PEG.
```

91/345

CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n, v-y = 0.

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H₄N₂-PEG

a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n = 0; v-y (independently selected) = 0 or 1; R = PEG.

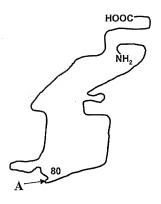
FIG. 29N

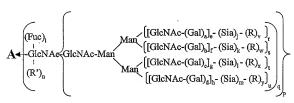
CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n, v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, r-u, z (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; n, v-y (independently selected) = 0.

FIG. 290





a-d, i, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group; R' = H, glycosyl group, modifying group, glycoconjugate.

```
\begin{split} &\text{Insect cell expressed Ifn-beta.} \\ &\text{a-d, f, h, j-m, s, u, v-y} = 0; \\ &\text{e, g, i, q, r, } t \text{ (independently selected)} = 0 \text{ or } 1. \end{split}
```

```
    GNT's 1,2,4,5, UDP-GlcNAc
    Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 29Q

```
Yeast expressed Ifn-beta. 
a-m=0; q-y (independently selected) = 0 to 1; p=1; R (branched or linear) = Man, oligomannose.
```

Endoglycanase
 Galactosyltransferase, UDP-Gal
 CMP-SA-PEG, ST3Gal3

```
a-m, p-y=0;
n (independently selected) = 0 or 1;
R'=-Gal-Sia-PEG.
```

FIG. 29R

94/345

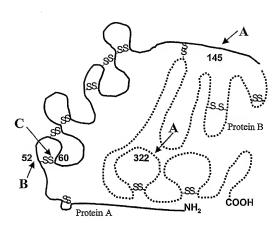
CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

- CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal3, desialylated transferrin.
 - 3. CMP-SA, ST3Gal3

a-m, q-u (independently selected) = 0 or 1; p=1; n=0; v-y (independently selected) = 0 or 1; R=linker-transferrin.

FIG. 29S

95/345



$$\mathbf{A} \longleftarrow \begin{bmatrix} [\mathrm{GlcNAc\text{-}(Gal)}_a]_{\mathfrak{g}^-} \cdot (\mathrm{Sia})_{\mathfrak{g}^-} \cdot (\mathrm{R})_v \\ [\mathrm{GlcNAc\text{-}Gal)}_b]_{\mathfrak{g}^-} \cdot (\mathrm{Sia})_{\mathfrak{g}^-} \cdot (\mathrm{R})_v \end{bmatrix}_{\mathfrak{g}}$$

$$\mathbf{B} \leftarrow \left\{ \text{Glc-}(Xyl)_n \right\}_n$$

a-d, i, q-u (independently selected) = 0 or 1. o, p (independently selected) = 0 or 1. e-h, n (independently selected) = 0 to 6. j-m (independently selected) = 0 to 20. v-y = 0; R = modifying group, mannose, oligomannose, Sia-Lewis X. Sia-Lewis A..

FIG. 30A

96/345

BHK expressed Factor VII or VIIa a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1; r, t = 1; f, h, k, m, s, u, v-y = 0; n = 0-4.

```
    Sialidase
    CMP-SA-PEG (16 mole eq),
ST3Gal3
```

```
a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1; r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4; v, x, (independently selected) = 1, when j, l (respectively, independently selected) is 1; R = PEG.
```

FIG. 30B

CHO, BHK, 293 cells, Vero expressed Factor VII or VIIa a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1; r, t=1; f, h, k, m, s, u, v-y=0; n=0-4.

```
    Sialidase
    CMP-SA-PEG (1.2 mole eq),
ST3Gal3
    CMP-SA (8 mol eq), ST3Gal3
```

```
a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1; r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4; v or x, (independently selected) = 1, when j or 1, (respectively, independently selected) is 1; R = PEG.
```

FIG. 30C

97/345

```
NSO expressed Factor VII or VIIa
a--u (independently selected) = 0 or 1;
v-y = 0; n = 0-4;
Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α -galactosidase 2. Galactosyltransferase, UDP-Gal
- I 3. CMP-SA-PEG, ST3Gal3

```
a-m, o-u (independently selected) = 0 or 1;

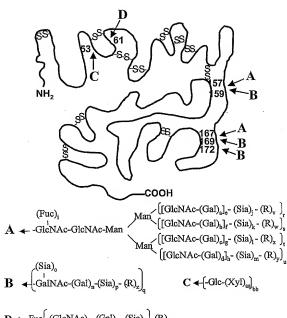
n = 0-4; v-y (independently selected) = 1,

when j-m (independently selected) is 1;

Sia = Sia; R = PEG.
```

FIG. 30D

98/345



D ←-Fuc (GlcNAc)_{cc}-(Gal)_{dd}-(Sia)_{ee} -(R)_{gg}

a-d, i, n-u (independently selected) = 0 or 1. bb, cc, dd, ee, ff, gg (independently selected) = 0 or 1. e-h, aa (independently selected) = 0 to 6. j-m (independently selected) = 0 to 20. v-z = 0: R = modifying group, mannose, oligo-mannose,

FIG. 31A

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q=1; e-h=1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg=0.
```

Sialidase
 CMP-SA-PEG, ST3Gal3

```
\
```

```
a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)
= 0 or 1;
o, p, z = 0;
j-m, ee, v-y, gg (independently selected) = 0 or 1;
R = PEG.
```

FIG. 31B

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, n, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

- Sialidase
 - 2. CMP-SA-PEG, ST3Gal3
 - 3. ST3Gal1, CMP-SA

```
a-d, n, p, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, r-u (independently selected) = 0 or 1;
j-m, ee, v-y, gg (independently selected) = 0 or 1;
o, z = 0; R = PEG.
```

FIG. 31C

100/345

CHO, BHK, 293 cells, Vero expressed Factor IX a-d, n, q, bb, cc, dd, ff = 1; e-h, aa = 1 to 4; ee, j-m, i, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.

- sialidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA, ST3Gal3
- 4. CMP-SA-PEG, ST3Gal1

```
a-d, n, q=1; e-h=1 to 4;
aa, bb, cc, dd, ee, ff, i, r-u (independently selected) = 0 or 1; R=PEG;
o, v-y, gg = 0;
j-m, p, ee (independently selected) = 0 or 1, but when p=1, z=1.
```

FIG. 31D

CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q=1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.

. CMP-SA-PEG, ST3Gal3

a-d, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected) = 0 or 1; R = PEG; o, p, z = 0; j-m, ee, v-y, gg (independently selected) = 0 or 1.

FIG. 31E

101/345

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

buffer, salt
2. H₄N₂-PEG

```
a-d, q=1; e-h=1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)
= 0 or 1;
o, p, z=0; R=PEG;
j-m, ee, v-y, gg (independently selected) = 0 or 1.
```

FIG. 31F

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, n, q, bb, ce, dd, ff = 1; e-h, aa = 1 to 4; ee, j-m, i, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

1. CMP-SA-PEG, α 2,8-ST

```
a-d, q = 1; e-h = 1 to 4;

aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)

= 0 or 1;

o, p, z = 0; R=PEG;

j-m, ee (independently selected) = 0 to 2;

v-y, gg (independently selected) = 1, when j-m

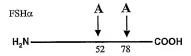
(independently selected) is 2;
```

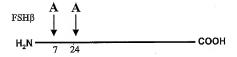
FIG. 31G

PCT/US02/32263

102/345

WO 03/031464





$$\mathbf{A} \leftarrow \underbrace{\left([\operatorname{GlcNAc-(Gal)}_{a}]_{s^{-}} (\operatorname{Sia})_{j} - (R)_{v} \right)_{r}^{T}}_{\left[[\operatorname{GlcNAc-(Gal)}_{b}]_{r} - (\operatorname{Sia})_{k} - (R)_{w} \right]_{s}^{T}}_{\left[[\operatorname{GlcNAc-(Gal)}_{d}]_{b}]_{r} - (\operatorname{Sia})_{k} - (R)_{w} \right]_{s}^{T}}_{\left[[\operatorname{GlcNAc-(Gal)}_{d}]_{h} - (\operatorname{Sia})_{m} - (R)_{y} \right]_{u}} \left(\underbrace{\left[[\operatorname{GlcNAc-(Gal)}_{d}]_{h} - (\operatorname{Sia})_{m} - (R)_{y} \right]_{u}}_{\left[[\operatorname{GlcNAc-(Gal)}_{d}]_{h} - (\operatorname{Sia})_{m} - (R)_{y} \right]_{u}}_{\left[[\operatorname{GlcNAc-(Gal)}_{d}]_{h} - (\operatorname{Sia})_{m} - (R)_{y} \right]_{u}} \left(\underbrace{\left[\operatorname{GlcNAc-(Gal)}_{d}]_{h} - (\operatorname{Sia})_{m} - (R)_{y} \right]_{u}}_{\left[[\operatorname{GlcNAc-(Gal)}_{d}]_{h} - (\operatorname{Sia})_{m} - (R)_{y} \right]_{u}}_{\left[[\operatorname{GlcNAc-(Gal)}_{d}]_{h} - (\operatorname{Sia})_{m} - (R)_{y} \right]_{u}} \left(\underbrace{\left[\operatorname{GlcNAc-(Gal)}_{d}]_{h} - (\operatorname{Sia})_{m} - (R)_{y} \right]_{u}}_{\left[[\operatorname{GlcNAc-(Gal)}_{d}]_{h} - (\operatorname{Sia})_{m} - (R)_{y} \right]_{u}}_{\left[\operatorname{GlcNAc-(Gal)}_{d}]_{h}} \left(\underbrace{\operatorname{GlcNAc-(Gal)}_{d}}_{\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h}}_{\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h}} \left(\underbrace{\operatorname{GlcNAc-(Gal)}_{d}}_{\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h}}_{h} \left(\underbrace{\operatorname{GlcNAc-(Gal)}_{d}}_{h} \right)_{h}}_{h} \right) \right)$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose.

FIG. 32A

PCT/US02/32263 WO 03/031464

103/345

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1: v-v = 0.

1. Sialidase

2. CMP-SA-PEG (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1: v-v (independently selected) = 1, when i-m (independently selected) is 1; R = PEG.

FIG. 32B

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, g-u (independently selected) = 0 or 1; e-h=1: v-v=0.

- Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3, CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y (independently selected) = 0 or 1; R = PEG.

104/345

NSO expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0; Sia (independently selected) = Sia or Gal.

- Sialidase and α-galactosidase
 Galactosyltransferase, UDP-Gal
 CMP-SA-PEG, ST3Gal1
- a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 1, when j-m (independently selected) is 1; R = PEG.

FIG. 32D

```
CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

 Sialidase
 CMP-SA-PEG (16 mol eq), ST3Gal3
 CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 32E

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H,N,-PEG

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 32F

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 32G

106/345

```
Insect cell expressed FSH.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

```
    GNT's 1,2,4,5, UDP-GlcNAc
    Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1;
j-m=0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R=PEG.
```

FIG. 32H

```
Yeast expressed FSH.

a-m=0; q-y (independently selected) = 0 to 1;

p=1;

R (branched or linear) = Man, oligomannose.
```

- Endoglycanase
 Galactosyltransferase, UDP-Gal
 CMP-SA-PEG, ST3Gal3
- a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.

107/345

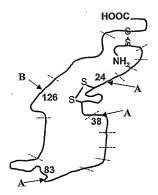
CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

 CMP-SA-linker-SA-CMP, ST3Gal3
 ST3Gal1, desialylated chorionic gonadrophin (CG) produced in CHO.
 CMP-SA, ST3Gal3, ST3Gal1

a-m, q-u (independently selected) = 0 or 1; p = 1; n = 0; v-y (independently selected) = 0 or 1; R = linker-CG.

FIG. 32J

108/345



$$\begin{array}{c} (\operatorname{Fuc})_{i} \\ \mathbf{A} \leftarrow -\operatorname{GlcNAc-GlcNAc-Man} \\ & \left[(\operatorname{GlcNAc-(Gal)}_{\mathfrak{g}}|_{\mathfrak{g}^{-}} (\operatorname{Sia})_{\mathfrak{f}^{-}} (\operatorname{R})_{\mathfrak{g}} \right]_{\mathfrak{g}} \\ & \left[(\operatorname{GlcNAc-(Gal)}_{\mathfrak{g}}|_{\mathfrak{g}^{-}} (\operatorname{Sia})_{\mathfrak{f}^{-}} (\operatorname{R})_{\mathfrak{g}} \right]_{\mathfrak{g}} \\ & \left[(\operatorname{GlcNAc-(Gal)}_{\mathfrak{g}}|_{\mathfrak{g}^{-}} (\operatorname{Sia})_{\mathfrak{f}^{-}} (\operatorname{R})_{\mathfrak{g}} \right]_{\mathfrak{g}} \\ & \left[(\operatorname{GlcNAc-(Gal)}_{\mathfrak{g}}|_{\mathfrak{g}^{-}} (\operatorname{Sia})_{\mathfrak{g}^{-}} (\operatorname{R})_{\mathfrak{g}} \right]_{\mathfrak{g}} \\ & \left[(\operatorname{GlcNAc-(Gal)}_{\mathfrak{g}}|_{\mathfrak{g}^{-}} (\operatorname{Sia})_{\mathfrak{g}^{-}} (\operatorname{R})_{\mathfrak{g}} \right]_{\mathfrak{g}} \\ & \left[(\operatorname{GlcNAc-(Gal)}_{\mathfrak{g}}|_{\mathfrak{g}^{-}} (\operatorname{Sia})_{\mathfrak{g}^{-}} (\operatorname{R})_{\mathfrak{g}} \right]_{\mathfrak{g}} \\ & \left[(\operatorname{GlcNAc-(Gal)}_{\mathfrak{g}^{-}} (\operatorname{Sia})_{\mathfrak{g}^{-}} (\operatorname{R})_{\mathfrak{g}^{-}} (\operatorname{R})_{\mathfrak{g}} \right]_{\mathfrak{g}} \\ & \left[(\operatorname{GlcNAc-(Gal)}_{\mathfrak{g}^{-}} (\operatorname{Sia})_{\mathfrak{g}^{-}} (\operatorname{R})_{\mathfrak{g}^{-}} (\operatorname{R})_$$

a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. v-z=0; R=modifying group, mannose, oligo-mannose.

FIG. 33A

109/345

```
CHO, BHK, 293 cells, Vero expressed EPO a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; v-z=0
```

Sialidase
 CMP-SA-PEG, ST3Gal3

```
a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
R = PEG; z = 0.
```

FIG. 33B

```
Insect cell expressed EPO
a-d, f, h, j-q, s, u, v-z = 0;
e, g, i, r, t (independently selected) = 0 or 1.
```

GNT's 1&2, UDP-GlcNAc
 Galactosyltransferase, UDP-Gal
 CMP-SA-PEG, ST3Gal3

```
b, d, f, h, k, m-q, s, u, w, y, z=0;
a, c, e, g, i, r, t (independently selected)= 0 or 1;
j, l, v, x (independently selected) = 0 or 1;
R=PEG.
```

FIG. 33C

110/345

```
CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z=0.
```

- sialidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA, ST3Gal3
- ↓ 4. CMP-SA-PEG, ST3Gal1

```
a-h, n, q = 1;
i-m, o, r-u (independently selected) = 0 or 1;
v-y = 0; p, z = 0 or 1; R = PEG.
```

FIG. 33D

```
CHO, BHK, 293 cells, Vero expressed EPO a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; v-z = 0
```

1. CMP-SA-PEG, ST3Gal3

```
a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 to 1;
j-m, v-y (independently selected) = 0 or 1;
R = PEG; z = 0.
```

FIG. 33E

111/345

Insect cell expressed EPO a-d, f, h, j-m, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

GNT's 1, 2 & 5, UDP-GlcNAc
 Galactosyltransferase, UDP-Gal-PEG

a-c, e-g, n, q-t, v-x, z (independently selected) = 0 or 1; d, h, j-m, o, p, y, z = 0; R = PEG.

FIG. 33F

Insect cell expressed EPO a-d, f, h, j-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT's 1, 2 & 5, UDP-GlcNAc 2. Galactosidase (synthetic enzyme),
- 2. Galactosidase (synthetic enzyme) PEG-Gal-F.

a-c, e-g, n, q-t, v-x, z (independently selected) = 0 or 1; d, h, j-m, o, p, y, z = 0; R = PEG.

FIG. 33G

112/345

```
NSO expressed NESP q=1; a-i, n, r-u (independently selected) = 0 or 1; j-m, o, p, v-z = 0
```

```
    CMP-SA-levulinate, ST3Gal3,
buffer, salt
    H<sub>4</sub>N<sub>7</sub>-PEG
```

```
q = 1; a-i, j-n, r-y (independently selected) = 0 or 1;
o, p, z = 0; R = PEG.
```

FIG. 33H

```
CHO, BHK, 293 cells, Vero expressed NESP a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; v-z=0
```

1. CMP-SA-PEG, α2,8-ST

```
a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 to 1;
j-m (independently selected) = 0 to 2;
v-y (independently selected) = 1,
when j-m (independently selected) is 2;
R = PEG; z = 0.
```

FIG. 331

113/345

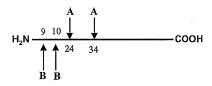
CHO, BHK, 293 cells, Vero expressed NESP a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 to1; v-z = 0

1. CMP-SA, poly-α2,8-ST

a-g, n, q = 1; h = 1 to 3; i, j-m, o, p, r-u, (independently selected) = 0 or 1; v-z (independently selected) = 0-40; R = Sia.

FIG. 33J

114/345



$$\begin{array}{c} \boldsymbol{A} & \longleftarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{s}]_{e^{-}} (\operatorname{Sia})_{j} - (\operatorname{R})_{v} \\ [\operatorname{GlcNAc-GlcNAc-Man} \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{s}]_{e^{-}} (\operatorname{Sia})_{j} - (\operatorname{R})_{v} \\ [\operatorname{GlcNAc-(Gal)}_{o}]_{g} - (\operatorname{Sia})_{l} - (\operatorname{R})_{x} \\ [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \\ \end{pmatrix}_{l} \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{e^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \\ \end{bmatrix}_{l} \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \\ [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \\ \end{bmatrix}_{l} \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \\ \end{bmatrix}_{l} \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ \end{bmatrix}_{l} \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ \end{bmatrix}_{l} \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Sia})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Gal})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Gal})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Gal})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Gal})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Gal})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Gal})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Gal})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Gal})_{l} - (\operatorname{Gal})_{o} \\ \\ & \longrightarrow \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_{o}]_{h^{-}} (\operatorname{Gal})_{l} - (\operatorname{Gal}$$

$$\mathbf{B} \leftarrow \begin{bmatrix} (\mathrm{Sia})_{\mathrm{o}} \\ -\mathrm{GaINAc-(GaI)_{n}-(Sia)_{p}-(R)_{z}} \end{bmatrix}_{\mathrm{aa}}$$

a-d, i, n-u, as (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer, glycoconjugate.

FIG. 34A

115/345

```
CHO, BHK, 293 cells, Vero expressed GM-CSF.
a-d, i-m, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; v-z = 0.

1. Sialidase
2. CMP-SA-PEG (16 mol eq),
```

ST3Gal3

```
a-d, i-m, q-u, aa (independently selected) = 0 or 1;
o, p, z = 0; n, e-h = 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 34B

```
CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z=0.
```

```
1. Sialidase
2. CMP-SA-PEG (1.2 mol eq),
ST3Gal3

▼ 3. CMP-SA (16 mol eq), ST3Gal3 &
ST3Gal1
```

```
a-d, i-m, p-u, aa (independently selected) = 0 or 1;
o, z = 0; n, e-h = 1;
v-y (independently selected) = 0 or 1; R = PEG.
```

FIG. 34C

```
NSO expressed GM-CSF.
a-d, i-m, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; v-z = 0;
Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α-galactosidase
- CMP-SA, ST3Gal3
- CMP-SA-PEG, ST3Gal1

```
a-d, i-m, p-u, z, as (independently selected) = 0 or 1; n, e-h = 1; o, v-y = 0; z = 1, when p = 1; R = PEG.
```

FIG. 34D

```
CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.
```

- 1. Sialidase
- CMP-SA-PEG (16 mol eq), ST3Gal3
 - 3. CMP-SA, ST3Gal3

a-d, i-m, q-y, as (independently selected) = 0 or 1; o, p, z = 0; n, e-h = 1; R = PEG.

FIG. 34E

117/345

CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h=1; v-z=0.

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H₄N₇-PEG

a-d, i-m, o-y, aa (independently selected) = 0 or 1; z = 0; n, e-h = 1; R = PEG.

FIG. 34F

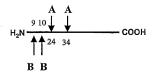
CHO, BHK, 293 cells, Vero expressed GMCSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z=0.

1. CMP-SA, α2,8-ST

a-d, i, o-u, as (independently selected) = 0 or 1; n, e-h = 1; j-m (independently selected) = 0-20; v-z (independently selected) = 0.

FIG. 34G

118/345



$$\mathbf{A} \overset{[[GlcNAc-(Gal)_a]_c^- (Sia)_j^- (R)_v]_r}{[[GlcNAc-(Gal)_b]_r^- (Sia)_k^- (R)_w]_s} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (Sia)_k^- (R)_w]_s}{[[GlcNAc-(Gal)_c]_g^- (Sia)_l^- (R)_x]_t} \\ \mathbf{Man} \overset{[[GlcNAc-(Gal)_d]_r^- (Sia)_m^- (R)_y]_u}{[[GlcNAc-(Gal)_d]_h^- (Sia)_m^- (R)_y]_u} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (Sia)_l^- (R)_w]_s}{[[GlcNAc-(Gal)_d]_h^- (Sia)_m^- (R)_y]_u} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (Sia)_l^- (R)_w]_s}{[[GlcNAc-(Gal)_d]_h^- (Sia)_m^- (R)_y]_u} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (Sia)_l^- (R)_w]_s}{[[GlcNAc-(Gal)_d]_h^- (Sia)_m^- (R)_w]_u} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (Sia)_l^- (R)_w]_s}{[[GlcNAc-(Gal)_b]_h^- (Sia)_m^- (R)_w]_u} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (Sia)_l^- (R)_w]_s}{[[GlcNAc-(Gal)_b]_h^- (Sia)_m^- (R)_w]_u} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (R)_w]_s}{[[GlcNAc-(Gal)_b]_h^- (R)_w]_u} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (R)_w]_s}{[[GlcNAc-(Gal)_b]_h^- (R)_w} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (R)_w]_s}{[[GlcNAc-(Gal)_b]_h^- (R)_w]_u} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (R)_w]_s}{[[GlcNAc-(Gal)_b]_h^- (R)_w} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (R)_w}{[[GlcNAc-(Gal)_b]_h^- (R)_w} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (R)_w}{[GlcNAc-(Gal)_w]_w} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (R)_w}{[GlcNAc-(Gal)_w]_w} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^- (R)_w}{[GlcNAc-(Gal)_w]_w} \\ \mathbf{A} \overset{[[GlcNAc-(Gal)_b]_r^$$

a-d, i, n-u, aa, bb, cc (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose. R'= H, glycosyl residue, modifying group, glycoconjugate.

FIG. 34H

119/345

```
Insect cell expressed GM-CSF.
a-d, f, h, j-m, o, p, s, u, v-z = 0;
e, g, i, n, q, r, t, aa (independently selected) = 0 or 1.
```

```
1. GNT's 1,2,4,5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, n, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 341

```
Yeast expressed GM-CSF.
a-p, z, &c = 0;
q-y, aa (independently selected) = 0 to 1;
bb = 1; R (branched or linear) = Man, oligomannose;
GalNAc = Man.
```

- 1. Endoglycanase
- 2. mannosidase (if aa = 1).
- 3. Galactosyltransferase, UDP-Gal-PEG

```
a-p, r-z, aa, bb = 0;
q, cc (independently selected) = 0 or 1;
R' = -Gal-PEG.
```

FIG. 34J

120/345

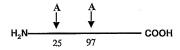
CHO, BHK, 293 cells, Vero expressed GM-CSF. a--m, o-u, aa, bb (independently selected) = 0 or 1; n, v-z, cc = 0.

- 1. sialidase
- CMP-SA, ST3Gal3
 CMP-SA-linker-SA-CMP, ST3Gal1
 ST3Gal3, transferrin

a--m, p-u, z, aa (independently selected) = 0 or 1; o, v-y, cc = 0; bb, n = 1; R = transferrin.

FIG. 34K

121/345



$$\mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc}\text{-}(\mathrm{Gal})_a]_{\mathfrak{g}^-}(\mathrm{Sia})_{\mathfrak{f}^-}(\mathrm{Ria})_{\mathfrak{$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 35A

122/345

```
CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
    Sialidase
    CMP-SA-PEG (16 mol eq),
ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 35B

```
CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
    Sialidase
    CMP-SA-PEG (1.2 mol eq),
ST3Gal3
    CMP-SA (16 mol eq), ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 35C

123/345

```
NSO expressed Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0;
Sia (independently selected) = Sia or Gal.
```

```
1. Sialidase and α-galactosidase
2. α-Galactosyltransferase, UDP-Gal

3. CMP-SA-PEG, ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 35D

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

```
    Sialidase
    CMP-SA-PEG (16 mol eq),
ST3Gal3
    CMP-SA, ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 35E

124/345

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA-levulinate, ST3Gal3,
2. H<sub>4</sub>N<sub>2</sub>-PEG

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 35F

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST
```

```
a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-20;
v-y (independently selected) = 0.
```

FIG. 35G

PCT/US02/32263

125/345



$$\mathbf{A} \bullet \begin{bmatrix} (\operatorname{Fuc})_i \\ - (\operatorname{GlcNAc-(Gal)_a}]_e - (\operatorname{Sia})_j - (\operatorname{R})_v \end{bmatrix}_r \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_b}]_f - (\operatorname{Sia})_k - (\operatorname{R})_w \end{bmatrix}_e \\ (\operatorname{R}')_n \\ \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_g - (\operatorname{Sia})_l - (\operatorname{R})_x \end{bmatrix}_t \\ \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Sia})_m - (\operatorname{R})_y \end{bmatrix}_u \\ = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)_a}]_h - (\operatorname{Gal)_a}]_h - (\operatorname{Gal)_a}_h - (\operatorname{Gal)_a}_h$$

a-d, i, n, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 35H

126/345

```
Insect or fungi cell expressed IF-gamma.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

```
1. GNT's 1,2,4,5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 351

```
Yeast expressed IF-gamma. 
a-m=0; q-y (independently selected) = 0 to 1; p=1; R (branched or linear) = Man, oligomannose.
```

- Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.
```

FIG. 35J

127/345

```
CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

 CMP-SA-linker-Gal-UDP, ST3Gal3
 Galactosyltransferase, transferrin treated with endoglycanase.

```
a-m, q-u (independently selected) = 0 or 1;
p = 1; n = 0;
v-y (independently selected) = 0 or 1;
R = linker-transferrin.
```

FIG. 35K

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h, p = 1; n, v-y = 0.

1. CMP-SA-PEG,
```

ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h, p = 1;
n, v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 35L

128/345

```
Insect or fungi cell expressed IF-gamma.

a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.

1. GNT's 1 & 2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, q (independently selected) = 0 or 1;
p = 1; v, x (independently selected) = 1,
when e, g (independently selected) is 1;
R = PEG.
```

FIG. 35M

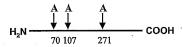
```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA-PEG, α2,8-ST
```

```
a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-2;
v-y (independently selected) = 1,
when j-m (independently selected) = 2;
R = PEG.
```

FIG. 35N

129/345



$$\mathbf{A} \leftarrow \underbrace{\left([\mathrm{GlcNAc} - (\mathrm{Gal})_{a}]_{s} - (\mathrm{Sia})_{j} - (\mathrm{R})_{v} \right)_{r}}_{\left[[\mathrm{GlcNAc} - (\mathrm{Gal})_{b}]_{s} - (\mathrm{Sia})_{t} - (\mathrm{R})_{w} \right]_{s}}_{\left[[\mathrm{GlcNAc} - (\mathrm{Gal})_{d}]_{s} - (\mathrm{Sia})_{t} - (\mathrm{R})_{x} \right]_{t}}_{\left[[\mathrm{GlcNAc} - (\mathrm{Gal})_{d}]_{h} - (\mathrm{Sia})_{m} - (\mathrm{R})_{y} \right]_{u}} \left([\mathrm{GlcNAc} - (\mathrm{Gal})_{d}]_{h} - (\mathrm{Sia})_{m} - (\mathrm{R})_{y} \right)_{u}} \left([\mathrm{GlcNAc} - (\mathrm{Gal})_{d}]_{h} - (\mathrm{Sia})_{m} - (\mathrm{R})_{y} \right)_{u}} \right)_{u} \left([\mathrm{GlcNAc} - (\mathrm{Gal})_{d}]_{h} - (\mathrm{Sia})_{m} - (\mathrm{R})_{y} \right)_{u}} \right)$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

130/345

CHO, BHK, 293 cells, Vero or transgenic animal expressed α_1 antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

Sialidase
 CMP-SA-PEG (16 mol eq),
 ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 1, when j-m (independently selected) is 1; R = PEG.

FIG. 36B

CHO, BHK, 293 cells, Vero or transgenic animal expressed α_1 antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

 Sialidase
 CMP-SA-PEG (1.2 mol eq), ST3Gal3

3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 36C

131/345

```
NSO expressed \alpha_1-antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = \dot{1}; v-y = 0;
Sia (independently selected) = Sia or Gal.
```

1. Sialidase and α-galactosidase
2. α-Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 36D

```
CHO, BHK, 293 cells, Vero or transgenic animal expressed alpha-1 antitrypsin.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0.
```

```
    Sialidase
    CMP-SA-PEG (16 mol eq),
ST3Gal3
    CMP-SA, ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.
```

FIG. 36E

132/345

CHO, BHK, 293 cells, Vero or transgenic animal expressed α_1 -antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H₄N₂-PEG

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

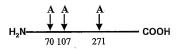
FIG. 36F

CHO, BHK, 293 cells, Vero expressed α_1 -antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-v = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

133/345



$$\mathbf{A} = \underbrace{\left([\operatorname{GlcNAc-(Gal)}_{a}]_{a^{-}} (\operatorname{Sia})_{j^{-}} (R)_{v} \right)_{r}}_{\left([\operatorname{GlcNAc-(Gal)}_{b}]_{a^{-}} (\operatorname{Sia})_{j^{-}} (R)_{w} \right)_{s}}_{\left([\operatorname{GlcNAc-(Gal)}_{b}]_{a^{-}} (\operatorname{Sia})_{j^{-}} (R)_{w} \right)_{s}}_{\left([\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (R)_{y} \right)_{u} \in \mathbb{R}^{d}}$$

a-d, i, n, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. y-y=0; R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 36H

134/345

Insect or fungi cell expressed α_1 -antitrypsin. a-d, f, h, j-m, s, u, v-y = 0; e, g, i, q, r, t (independently selected) = 0 or 1.

```
1. GNT's 1,2,4,5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1; j-m = 0; v-y (independently selected) = 1, when e-h (independently selected) is 1; R = PEG.
```

FIG. 361

$$\label{eq:continuous} \begin{split} & Yeast\ expressed\ \alpha_1\text{-antitrypsin.}\\ & a\text{-}m=0;\ q\text{-}y\ (\text{independently selected})=0\ \text{to}\ 1;\\ & p=1;\ R\ (\text{branched or linear})=\text{Man, oligomannose.} \end{split}$$

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-m, p-y=0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.

FIG. 36J

135/345

```
CHO, BHK, 293 cells, Vero expressed \alpha_1-antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- CMP-SA-linker-Gal-UDP, ST3Gal3
- 2. Galactosyltransferase, transferrin treated with endoglycanase

```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker-transferrin.
```

FIG. 36K

136/345



$$\begin{array}{c} \text{(Fuc)}_{i} \\ \textbf{A} \leftarrow \text{-GlcNAc-GlcNAc-Man} \\ \text{(R')}_{p} \\ \end{array} \\ \begin{array}{c} \text{(GlcNAc-(Gal)}_{s}]_{e^{-}} \left(\text{Sia)}_{j^{-}} \left(\text{Ria}\right)_{j^{-}} \left(\text{Ria}\right)_{j^{-}} \left(\text{Ria}\right)_{j^{-}} \\ \left[\left[\text{GlcNAc-(Gal)}_{c}\right]_{g^{-}} \left(\text{Sia}\right)_{l^{-}} \left(\text{Ria}\right)_{j^{-}} \left(\text{Ria}\right)_{j^{-}} \\ \left[\left[\text{GlcNAc-(Gal)}_{c}\right]_{g^{-}} \left(\text{Sia}\right)_{l^{-}} \left(\text{Ria}\right)_{j^{-}} \right]_{l^{-}} \\ \left[\left[\text{GlcNAc-(Gal)}_{c}\right]_{d^{-}} \left(\text{Sia}\right)_{m^{-}} \left(\text{Ria}\right)_{j^{-}} \\ \end{array} \\ \end{array}$$

a-d, i, n-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

R = polymer;

R', R" (independently selected) = sugar, glycoconjugate.

137/345

Yeast expressed alpha-1 antitrypsin. a-h, i-m, p, q = 0; R (independently selected) = mannose, oligomannose, polymannose; r-u, v-y (independently selected) = 0 or 1; n, o = 1.

- 1. endoglycanase
- ▼ 2. Galactosyltransferase, UDP-Gal-PEG

```
a-h, i-o, q, r-u, v-y = 0; p = 1.
R" = Gal-PEG.
```

FIG. 36M

Plant expressed alpha-1 antitrypsin. a-d, f, h, j-m, s, u , v-y = 0; e, g, i, q, r, t (independently selected) = 0 or 1; n=1; R'=xylose

- 1. hexosaminidase,
- 2. alpha mannosidase and xylosidase
- 3. GlcNAc transferase, UDP-GlcNAc-PEG

```
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0;
q = 1; R' = GlcNAc-PEG.
```

FIG. 36N

138/345

CHO, BHK, 293 cells, Vero, transgenic animal expressed α_1 antitrypsin. a-h, i-o, r-u (independently selected) = 0 or 1; p, q, v-y = 0.

 CMP-SA-PEG, ST3Gal3

a-h, i-o, r-u (independently selected) = 0 or 1; p, q = 0; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 360

139/345



$$\begin{array}{c} \text{(Fuc)}_{i} \\ \text{(Fuc)}_{i} \\ \text{(GlcNAc-(Gal)}_{a}]_{e}^{-} \text{(Sia)}_{j}^{-} \text{(R)}_{v} \\ \text{([GlcNAc-(Gal)}_{b}]_{r}^{-} \text{(Sia)}_{k}^{-} \text{(R)}_{w} \\ \text{([GlcNAc-(Gal)}_{a}]}_{g}^{-} \text{(Sia)}_{l}^{-} \text{(R)}_{x} \\ \text{([GlcNAc-(Gal)}_{d}]_{h}^{-} \text{(Sia)}_{m}^{-} \text{(R)}_{y} \\ \text{([GlcNAc-(Gal)}_{d}]_{h}^{-} \text{(Sia)}_{m}^{-} \text{(R)}_{y} \\ \text{([GlcNAc-(Gal)}_{d}]_{h}^{-} \text{(Sia)}_{m}^{-} \text{(R)}_{y} \\ \text{([GlcNAc-(Gal)}_{d}]_{h}^{-} \text{(Sia)}_{m}^{-} \text{(R)}_{y} \\ \text{(Sia)}_{m}^{-} \text{(R)}_{y} \\ \text{([GlcNAc-(Gal)}_{d}]_{h}^{-} \text{(Sia)}_{m}^{-} \text{(R)}_{y} \\ \text{(R)}_{m}^{-} \text{(R)}_{m}^{-} \text{(R)}_{m}^{-} \text{(R)}_{m}^{-} \\ \text{(R)}_{m}^{$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

140/345

```
CHO, BHK, 293 cells, Vero expressed Cerezyme a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y=0.
```

```
    Sialidase
    CMP-SA-PEG (16 mol eq),
ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 37B

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

```
    Sialidase
    CMP-SA-M-6-P (1.2 mol eq),
ST3Gal3
    CMP-SA (16 mol eq), ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = mannose-6-phosphate
```

FIG. 37C

141/345

```
NSO expressed Cerezyme.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.
```

1. Sialidase and α-galactosidase
2. α-Galactosyltransferase, UDP-Gal

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = mannose-6 phosphate
```

FIG. 37D

```
CHO, BHK, 293 cells, Vero expressed Cerezyme.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. Sialidase
2. CMP-SA-PEG (16 mol eq),
ST3Gal3
3. CMP-SA, ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = Mannose-6-phosphate
```

142/345

CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

```
    CMP-SA-levulinate, ST3Gal3,
buffer, salt
    H<sub>4</sub>N<sub>4</sub>-spacer-M-6-P or clustered M-6-P
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = M-6-P or clustered M-6-P
```

FIG. 37F

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
1. CMP-SA, α2,8-ST
```

```
a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-20;
v-y (independently selected) = 0.
```

FIG. 37G

143/345



$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} & & & & \\ (\operatorname{Fuc})_{i} & & & & \\ (\operatorname{GlcNAc-(Gal)}_{a}]_{e^{-}} (\operatorname{Sia})_{j^{-}} (R)_{v} & \\ (\operatorname{GlcNAc-(Gal)}_{b}]_{f^{-}} (\operatorname{Sia})_{k^{-}} (R)_{w} \end{bmatrix}_{s} \\ (R')_{n} & & & & \\ (\operatorname{GlcNAc-(Gal)}_{d}]_{g^{-}} (\operatorname{Sia})_{l^{-}} (R)_{x} & \\ (\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (R)_{y} \end{bmatrix}_{u} \\ q_{p} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{h^{-}} (\operatorname{Sia})_{m^{-}} (R)_{y} \end{bmatrix}_{u} \\ q_{p} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{h^{-}} (\operatorname{Sia})_{m^{-}} (R)_{y} \end{bmatrix}_{u} \\ q_{p} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{h^{-}} (\operatorname{Sia})_{m^{-}} (R)_{y} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{h^{-}} (\operatorname{Gal})_{m^{-}} (R)_{y} \\ (\operatorname{Gal})_{m^{-}}$$

a-d, i, n, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

144/345

```
Insect cell expressed Cerezyme.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

```
1. GNT's 1,2,4,5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0;
v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 371

```
Yeast expressed Cerezyme. 
a-m = 0; q-y (independently selected) = 0 to 1; 
p = 1; R (branched or linear) = Man, oligomannose.
```

- Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- ▼ 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0; n (independently selected) = 0 or 1;
R' = -Gal-Sia-PEG.
```

FIG. 37J

145/345

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
1. CMP-SA-linker-SA-CMP,
ST3Gal3
2. ST3Gal3, desialylated transferrin.
3. CMP-SA, ST3Gal3
```

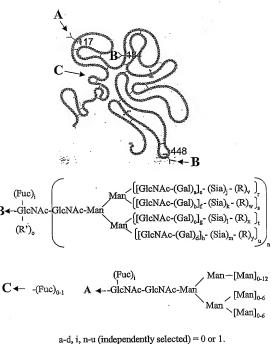
```
a-m, q-u (independently selected) = 0 or 1; p = 1; n = 0; v-y (independently selected) = 0 or 1; R = linker-transferrin.
```

FIG. 37K

PCT/US02/32263

146/345

WO 03/031464



a-d, 1, n-u (independently selected) = 0 or 1 e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. R = polymer; R' = sugar, glycoconjugate.

FIG. 38A

147/345

```
CHO, BHK, 293 cells, Vero expressed tPA a-g, n=1; h=1 to 3; j-m, i, (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; o, v-y = 0.
```

Mannosidase(s), sialidase
 GNT1,2 (4 and/or 5) UDP-GlcNAc
 Gal transferase, UDP-Gal

↓ 4. CMP-SA-PEG, ST3Gal3

```
A = B; a-g, n = 1; h = 1 to 3;
i, r-u (independently selected) = 0 or 1;
o = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG
```

FIG. 38B

```
Insect or fungi cell expressed tPA A=B;\ a-d,\ f,\ h,\ j-o,\ s,\ u,\ v-y=0; e, g, i, n, r, t (independently selected) = 0 or 1.
```

1. GNT's 1&2, UDP-GlcNAc 2. Galactosyltransferase, UDP-Gal 3. CMP-SA-PEG, ST3Gal3

 $A=B; \ b,d,\ f,\ h,\ k,m,o,s,u,w,y=0;$ a, c, e, g, i, r, t (independently selected) = 0 or 1; n=1; j,l,v,x (independently selected) = 0 or 1; R=PEG.

FIG. 38C

148/345

Yeast expressed tPA B = A; i = 0.

- endoglycanase
 Galactosyltransferase,
- UDP-Gal-PEG

A = B; a-n, r-y = 0; o = 1; R' = Gal-PEG.

FIG. 38D

Insect or fungi cell expressed tPA

A = B; a-d, f, h, j-o, s, u, v-y = 0;

e, g, i, n, r, t (independently selected) = 0 or 1.

- 1, alpha and beta mannosidases
- 2. Galactosyltransferase, UDP-Gal-PEG

A = B; a-n, r-y = 0; o = 1; R' = Gal-PEG.

FIG. 38E

149/345

Insect or fungi cell expressed tPA $A=B;\ a-d,\ f,\ h,\ j-o,\ s,\ u,\ v-y=0;$ e, g, i, n, r, t (independently selected) = 0 or 1.

- GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

A=B; b, d, f, h, j-o, s, u, w, y = 0; a, c, e, g, i, r, t, v, x (independently selected)= 0 or 1; n=1; R=PEG.

FIG. 38F

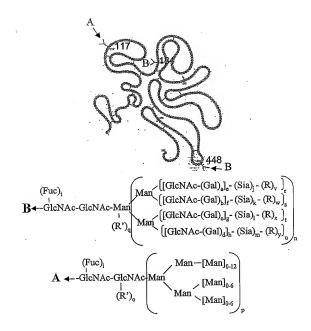
Insect or fungi cell expressed tPA A=B; a-d, f, h, j-o, s, u, v-y = 0; e, g, i, n, r, t (independently selected) = 0 or 1.

- 1. GNT's 1 & 2, UDP-GlcNAc
- 2. Galactosidase (synthetic enzyme), PEG-Gal-F.

 $\begin{array}{lll} A=B; & b,d, \ f, \ h, \ j\text{-o}, s,u,w,y=0;\\ a,c,e,g, \ i, \ r, \ t,v,x \ (independently \ selected)=0 \ or \ 1;\\ n=1; \ R=PEG. \end{array}$

FIG. 38G

150/345



a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. R = polymer; R' = sugar, glycoconjugate.

151/345

```
NSO expressed tPA

A = B; a-m, r-u (independently selected) = 0 or 1;

n = 1; o, p, q, v-y = 0
```

```
1. sialidase, alpha-galactosidase
2. CMP-SA-levulinate, ST3Gal3,
```

```
A = B; a-m, r-y (independently selected) = 0 or 1;

n = 1; o, p, q = 0;

v-y (independently selected) = 1,

when j-m (independently selected) is 1;

R = PEG.
```

FIG. 381

```
CHO, BHK, 293 cells, Vero expressed tPA a-g, n, p = 1; h = 1 to 3; j-m, i, (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; q, o, v-y = 0.
```

alpha and beta Mannosidases
 CMP-SA, ST3Gal3
 Galactosyltransferase, UDP-Gal-PEG

```
a-g, n = 1; h = 1 to 3;
i, r-u (independently selected) = 0 or 1; o = 1;
q, p, v-y = 0; j-m (independently selected) = 0 or 1;
R' = Gal-PEG
```

FIG. 38J

152/345

Plant expressed tPA

Than cospected 11 A = B; a - d, f, h, j - m, s, u, v - y = 0; e, g, f, g, f, f (independently selected) = 0 or 1; f f = f f = f = f f = f f = f f = f f = f f f = f f = f f = f f = f f = f f = f f = f f = f f = f f = f f =

- 1. hexosaminidase,
- 2. alpha mannosidase and

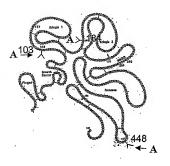
xylosidase

3. GlcNAc transferase, UDP-GlcNAc-PEG

A = B; a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t (independently selected) = 0; q = 1; R' = GleNAc-PEG.

FIG. 38K

153/345



$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} & \operatorname{Man} & \left[[\operatorname{GlcNAc-(Gal)}_{a}]_{e^{-}} & (\operatorname{Sia})_{j^{-}} & (\operatorname{R})_{v^{-}} \right]_{r} \\ - \operatorname{GlcNAc-GlcNAc-Man} & \left[[\operatorname{GlcNAc-(Gal)}_{a}]_{f^{-}} & (\operatorname{Sia})_{k^{-}} & (\operatorname{R})_{w^{-}} \right]_{s} \\ - \operatorname{Man} & \left[[\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} & (\operatorname{Sia})_{m^{-}} & (\operatorname{R})_{y^{-}} \right]_{u^{-}} \\ - \operatorname{GlcNAc-(Gal)}_{d} & \left[[\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} & (\operatorname{Sia})_{m^{-}} & (\operatorname{R})_{y^{-}} \right]_{u^{-}} \\ - \operatorname{GlcNAc-(Gal)}_{u^{-}} & \left[\operatorname{GlcNAc-(Gal)}_{u^{-}} & (\operatorname{Sia})_{m^{-}} & (\operatorname{R})_{y^{-}} \right]_{u^{-}} \\ - \operatorname{GlcNAc-(Gal)}_{u^{-}} & \left[\operatorname{GlcNAc-(Gal)}_{u^{-}} & (\operatorname{Sia})_{m^{-}} & (\operatorname{R})_{y^{-}} \right]_{u^{-}} \\ - \operatorname{GlcNAc-(Gal)}_{u^{-}} & \left[\operatorname{GlcNAc-(Gal)}_{u^{-}} & (\operatorname{Sia})_{m^{-}} & (\operatorname{R})_{y^{-}} \right]_{u^{-}} \\ - \operatorname{GlcNAc-(Gal)}_{u^{-}} & \left[\operatorname{GlcNAc-(Gal)}_{u^{-}} & (\operatorname{Sia})_{m^{-}} & (\operatorname{Sia})_{m^{-}} & (\operatorname{Sia})_{m^{-}} & (\operatorname{Sia})_{m^{-}} & (\operatorname{Sia})_{m^{-}} & (\operatorname{Sia})_{m^{-}} \\ - \operatorname{GlcNAc-(Gal)}_{u^{-}} & \left[\operatorname{GlcNAc-(Gal)}_{u^{-}} & (\operatorname{Sia})_{m^{-}} \\ - \operatorname{GlcNAc-(Gal)}_{u^{-}} & \left[\operatorname{GlcNAc-(Gal)}_{u^{-}} & (\operatorname{Sia})_{m^{-}} & (\operatorname{Sia})$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

154/345

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

 Sialidase
 CMP-SA-PEG (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 1, when j-m (independently selected) is 1; R = PEG.

FIG. 38M

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

 Sialidase
 CMP-SA-PEG (1.2 mol eq), ST3Gal3
 CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 38N

155/345

```
NSO expressed TNK tPA
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0;
Sia (independently selected) = Sia or Gal.
```

Sialidase and α-galactosidase

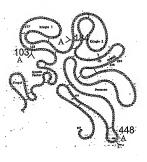
2. Galactosyltransferase, UDP-Gal

▼ 3. CMP-SA-PEG, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 1, when j-m (independently selected) is 1; R = PEG.

FIG. 380

156/345



$$\begin{array}{c} \text{(Fuc)}_{i} \\ \text{(Fuc)}_{i} \\ \text{(GlcNAc-(Gal)}_{b}]_{c} - (\text{Sia)}_{j} - (R)_{v} \\ \text{([GlcNAc-(Gal)}_{b}]_{f} - (\text{Sia)}_{k} - (R)_{w} \\ \text{([GlcNAc-(Gal)}_{d}]_{g} - (\text{Sia)}_{l} - (R)_{x} \\ \text{([GlcNAc-(Gal)}_{d}]_{h} - (\text{Sia})_{m} - (R)_{y} \\ \text{([GlcNAc-(Gal)}_{d}]_{h} - (\text{Sia})_{m} - (R)_{y} \\ \text{([GlcNAc-(Gal)}_{d}]_{h} - (\text{Sia})_{m} - (R)_{y} \\ \text{(Sia)}_{l} - (R)_{y} \\ \text{(Sia)}_{$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

157/345

```
CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
    Sialidase
```

- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3, CMP-SA, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 38Q

```
CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
1. CMP-SA-levulinate, ST3Gal3,
buffer, salt
2. H<sub>4</sub>N<sub>2</sub>-PEG
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

158/345

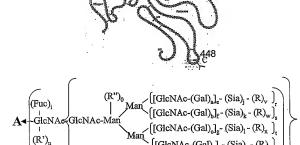
CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 38S

159/345



a-d, i, n-y (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

R" = glycosyl residue.

160/345

```
Insect cell expressed TNK tPA a-d, f, h, j-m, s, u, v-y=0; e, g, i, q, r, t (independently selected) = 0 or 1.
```

```
    GNT's 1,2,4,5, UDP-GlcNAc
    Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 38U

```
Yeast expressed TNK tPA a-m = 0; q-y (independently selected) = 0 to 1; p = 1; R (branched or linear) = Man, oligomannose.
```

```
    Endoglycanase
    Galactosyltransferase, UDP-Gal-PEG
```

```
a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal\text{-PEG}.
```

FIG. 38V

161/345

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

```
1. CMP-SA-linker-Gal-UDP,
ST3Gal3
2. Galactosyltransferase, anti-TNF
IG chimera produced in CHO.
```

a-m, r-u (independently selected) = 0 or 1; p, q = 1; n = 0; v-y (independently selected) = 0 or 1; R = linker-anti-TNF IG chimera protein.

FIG. 38W